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Prevalence of *Giardia duodenalis* assemblages in weaned cattle on cow-calf operations in the United States

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ABSTRACT

To determine the prevalence of *Giardia duodenalis* in weaned beef calves on cow-calf operations in the United States, fecal specimens were collected from 819 calves (6–18 months of age) from 49 operations. After cleaning and concentration procedures to maximize recovery of cysts from feces, DNA was extracted from each of the 819 specimens. The presence of *G. duodenalis* was determined by nested PCR of a fragment of the SSU rRNA gene. All positive PCR products were subjected to sequence analysis. The overall sample level prevalence of *Giardia* was 33.5% with prevalence ranging from 0 to 100% among operations. The highest within herd prevalence of infected beef calves was found in one cow-calf operation from the South region (100%), followed by a cow-calf operation from the West region (90%), and three cow-calf operations from the Midwest region (87.5, 85, and 85%). *Giardia* was not detected in samples from 7 operations including 5 cow-calf operations from the South region, and 1 cow-calf operation each from the Midwest and West regions. Molecular analysis of the *Giardia*-positive samples identified assemblage E (or E-like) in 31.7% of all samples (260/819) and assemblage A in 1.2% (10/819). A mixed infection with assemblages A and E was observed in four calves from an operation in Midwest region. The potentially zoonotic assemblage A was detected in specimens from four operations in Midwest region. These findings indicate that most *G. duodenalis* found in weaned beef calves was assemblage E which represents no known zoonotic threat. However, the presence of assemblage A in a small number of animals poses a potential risk of infection to humans.

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1. Introduction

Giardia duodenalis (syn. *Giardia lamblia*, *Giardia intestinalis*) is a widespread and prevalent intestinal parasite with a broad host range that includes humans, domestic animals, and wildlife. In humans, *G. duodenalis* is one of the most frequently identified parasites causing diarrhea worldwide (Cacciò et al., 2005). Understanding the role of animals in the epidemiology of human infections is essential to public health. Giardiasis in cattle is of particular concern because of the reported high prevalence of infection combined with the large output of feces, potentially leading to contamination of surface and ground water. The clinical signs associated with *G. duodenalis* infections in cattle can vary with subclinical infections often reported; however, infection can result in the onset of diarrhea, ill thrift and decreased weight gain in young calves (O'Handley et al., 1999; Geurden et al., 2006, 2010). Although point prevalence studies worldwide have detected considerable variation in the number of cattle excreting *Giardia* cysts (Xiao, 1994; Appelbee et al., 2003; Trout et al., 2004, 2005, 2006, 2007; Gow and Waldner, 2006; Hamnes et al., 2006;

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Castro-Hermida et al., 2007; Mendonça et al., 2007; Geurden et al., 2008; Hoar et al., 2009; Ng et al., 2011; Dixon et al., 2011), longitudinal studies have reported cumulative prevalences in cattle at 100% (Xiao and Herd, 1994; O'Handley et al., 1999; Ralston et al., 2003; Santín et al., 2009; Coklin et al., 2010).

Molecular characterization of *G. duodenalis* has revealed seven major genotypes (assemblages) with different host ranges (Monis et al., 2003; Thompson and Monis, 2004). Only assemblages A and B have been identified in humans, although A and B have been identified in a wide range of other mammalian hosts as well. Each of the remaining assemblages (C through G) appears host-specific. Recently, a novel assemblage in marine mammals (Assemblage H) has been suggested (Lasek-Nesselquist et al., 2010) as well as a novel genotype named quenda genotype (Adams et al., 2004; Ng et al., 2011). In cattle, three assemblages have been commonly detected, assemblages A, B, and E, with E most frequently reported followed by assemblage A (O'Handley et al., 2000; Huetink et al., 2001; Trout et al., 2004, 2005, 2006, 2007; Lalle et al., 2005; Coklin et al., 2007; Langkjaer et al., 2007; Geurden et al., 2008; Mendonça et al., 2007; Feng et al., 2008; Santín et al., 2009; Dixon et al., 2011); assemblage B has been reported in a few cattle in Australia, Italy, Canada, New Zealand, and Portugal (Lalle et al., 2005; Coklin et al., 2007; Mendonça et al., 2007; Winkworth et al., 2008; Ng et al., 2011; Dixon et al., 2011). The novel quenda genotype has been reported in one calf in Australia (Ng et al., 2011).

Much of the prevalence data of *Giardia* in cattle have been based on microscopic methods whereas prevalence data based on molecular studies are less readily available and have focused mainly on dairy cattle. Surveys of dairy cattle in Australia, Belgium, Brazil, Canada, Demark, Italy, Japan, the Netherlands, Spain and in the United States in which molecular data were obtained have reported a predominance of infections with assemblage E and few infections with assemblage A (O'Handley et al., 2000; Huetink et al., 2001; Trout et al., 2004; Berrilli et al., 2004; Itagaki et al., 2005; Uehlinger et al., 2006; Castro-Hermida et al., 2007; Langkjaer et al., 2007; Souza et al., 2007; Feng et al., 2008; Geurden et al., 2008; Santín et al., 2009). In four point prevalence studies of dairy cattle for *G. duodenalis* in the eastern United States involving approximately 2000 animals in four age categories, assemblage E was found in 34% of pre-weaned calves, 45% of post-weaned calves, 33% of heifers, and 25% of adults, whereas assemblage A was detected in 6% of pre-weaned calves, 7% of post-weaned calves, 3% of heifers, and 2% of adult cows (Trout et al., 2004, 2005, 2006, 2007). A longitudinal study involving 30 dairy cows from birth to 2 years of age on a dairy farm in the Unites States revealed the presence of assemblages E, A, and mixed infection of both A and E in 26.9, 4.4, 0.2% of the samples, respectively. In the same study the cumulative prevalence of assemblages E and A was found to be 100 and 70%, respectively (Santín et al., 2009).

Few studies have been conducted on the prevalence of *G. duodenalis* in beef cattle and most of them lack molecular data. As in dairy cattle, the reported prevalence of *Giardia* in beef cattle ranged widely (Appelbee et al., 2003; Ralston et al., 2003; McAllister et al., 2005; Gow and Waldner, 2006; Geurden et al., 2008; Hoar et al., 2009; Dixon et al., 2011). In Alberta, Canada 20 beef calves were examined from birth to weaning for the presence of *Giardia* cysts with the aid of immunofluorescence microscopy (Ralston et al., 2003). The cumulative prevalence was 100%. Also in Alberta, Canada fecal samples were examined for *G. duodenalis* cysts by immunofluorescence microscopy from 2- to 10-week-old beef calves on cow-calf farms (Appelbee et al., 2003). Cysts were found in 168 of the 495 fecal samples, with a prevalence of 7–60% of the calves among farms. On farms in western Canada, fecal samples collected from 560 beef cows and 605 calves were examined with the aid of a quantitative sucrose gradient immunofluorescent antibody test (Gow and Waldner, 2006). *Giardia* cysts were detected in feces from 17.0% of the cows and 22.6% of the calves. In the United States, 5260 fecal samples were collected from beef cattle in 22 feedlots in California, Colorado, Nebraska, Oklahoma, South Dakota, Texas, and Washington (Hoar et al., 2009). With the aid of immunofluorescence microscopy, *G. duodenalis* cysts were detected in 19.1% of samples, with prevalence ranging from 5.4 to 43.6% within feedlots.

Molecular characterization of *Giardia* isolates from beef cattle was conducted in a study in Ontario, Canada that included 112 pooled manure samples from 30 beef cattle farms. The overall prevalence of *Giardia* was 72% by IFA and 77% by PCR, and only assemblage E was identified (Dixon et al., 2011). Also in Canada, Appelbee et al. (2003) reported *Giardia* in 34% of the fecal samples from beef calves and genotypic analysis revealed a predominance of assemblage E in beef calves (41 out of the 42 samples); assemblage A was identified in only one sample. In Belgium, a survey that included 333 calves from 50 beef farms estimated the prevalence of *Giardia* to be 45%; once more assemblage E was predominant and few animals were infected with assemblage A (Geurden et al., 2008).

Based on the relatively small amount of prevalence data available for giardiasis in beef cattle and the greater lack of molecular data, the present study was undertaken to determine the prevalence of *G. duodenalis* in weaned beef calves in the United States.

2. Materials and methods

2.1. Cow-calf operations and sample processing

The USDA National Animal Health Monitoring System conducted the Beef 2007–2008 study on cow-calf operations in 24 states (USDA, 2008). Fecal samples for *Giardia* testing were available from operations in 20 of the 24 states from three regions (Midwest: Iowa, Kansas, Missouri, Nebraska, North Dakota, and South Dakota, South: Alabama, Georgia, Louisiana, Mississippi, Oklahoma, Tennessee, Texas, and Virginia and West: California, Colorado, Idaho, New Mexico, Oregon, and Wyoming). Participating producers each collected up to 20 fecal samples directly from the rectum of 6- to 18-month-old weaned calves or from fresh fecal pats on the ground from calves to assess parasite burden as described (Fayer et al., 2010). Specimens received at the Environmental Microbial and Food Safety Laboratory were examined for the presence of *G. duodenalis*

after cysts were concentrated from feces by sieving and CsCl density gradient centrifugation to remove fecal debris and concentrate cysts as described (Trout et al., 2004). Each 15 g sample of feces was suspended in 35-ml dH_2O and passed through a sieve with a 45 μm pore size and transferred to a 50 ml conical tube. After adjusting the volume to 50 ml with dH_2O the tube was centrifuged at 1800 × *g* for 15 min. The pellet was re-suspended in 25 ml dH_2O; 25 ml CsCl (1.4 specific gravity) was added, and the tube contents mixed by vortexing. The tube was centrifuged at 300 × *g* for 20 min and 4 ml of supernatant was aspirated from the surface. The aspirate was washed twice with dH_2O and the final pellet was suspended in 500 μl of dH_2O for subsequent DNA processing.

2.2. DNA extraction

DNA was extracted from each specimen using a DNeasy Tissue Kit (Qiagen, Valencia, CA). The slightly modified protocol, described below, employed reagents from the manufacturer. Fifty microliters of processed specimen was suspended in 180 μl of ATL buffer, thoroughly mixed, 20 μl of proteinase K (20 mg/ml) was added, and the suspension was mixed again. Following overnight incubation at 55 °C, 200 μl of AL buffer was added. The remaining protocol followed manufacturer's instructions except, to recover more DNA the nucleic acid was eluted in 100 μl of AE buffer.

2.3. PCR and DNA sequence analysis

A 292 bp fragment of the SSU-rRNA gene was amplified using nested PCR as previously described (Hopkins et al., 1997). PCR products were analyzed on 1% agarose gel stained with ethidium bromide. All positive products were purified with Exonuclease I/Shrimp Alkaline Phosphatase (Exo-SAP-IT™) (USB Corporation, Cleveland, OH) and sequenced in both directions with the same primers in 10 μl reactions, using Big Dye™ chemistries and an ABI 3130 sequencer analyzer (Applied Biosystems, Foster City, CA). Chromatograms of each strand were aligned and examined with Lasergene software (DNASTAR, Inc., Madison, WI).

The nucleotide sequences have been deposited in the GenBank database under accession numbers JN375979–JN375981.

3. Results

3.1. Prevalence of Giardia

Samples from 819 6–18 month-old weaned calves on 49 cow-calf operations from 3 regions of the United States (Midwest, South and West) were evaluated. The number and location of calves infected with *Giardia* determined by PCR are shown in Table 1. *G. duodenalis* was identified in 33.5% (274/819) of fecal samples, and 85.7% (42/49) of the cow-calf operations had at least one positive sample. In the Midwest region, 95% of operations (19/20) had at least one positive sample, compared with 93% in the West region (13/14) and 67% in the South region (9/15). The region with the highest prevalence was the Midwest region with 46% (155/337), followed by the West region (83/250). The lowest prevalence was found at the South region with a 15.5% (36/232). The highest prevalence of positive calves was at an operation in the South region (100%), followed by an operation on the West region (90%), and three in the Midwest region (85, 85, and 87.5%). *Giardia* was not detected in samples from 7 operations including 5 cow-calf operations from the South region, and 1 cow-calf operation each from the Midwest and West regions.

3.2. Molecular characterization

Sequence analysis of the 274 PCR-positive samples revealed the presence of assemblage E in 31.5% of samples (258/819), assemblage E-like in 0.2% samples (2/819), and assemblage A in 1.2% samples (10/819). Four samples revealed a mixture of A/E assemblages (0.5%), characterized by the presence of overlapping nucleotide peaks in chromatograms, indicating mixed infections with two assemblages in the same host. All 258 isolates identified as assemblage E showed 100% nucleotide sequence identity to the assemblage E reported by Trout et al. (2004) (Genbank accession number AY655701); all 10 isolates identified as assemblage A showed 100% nucleotide sequence identity to the assemblage A reported by Trout et al. (2004) (Genbank accession number AY655700); and the 2 isolates identified as assemblage E-like showed 99.7% nucleotide sequence identity to the assemblage E reported by Trout et al. (2004) (Genbank accession number AY655701).

4. Discussion

The results from this study suggest that weaned calves shed *G. duodenalis* at most cow-calf operations in the United States. The overall prevalence of *G. duodenalis* among weaned calves was 33.5% and the overall prevalence among operations was 85.7%. These prevalence figures most likely underestimate the actual prevalence of infection in these cattle because only one sample per animal was collected and cyst excretion can be intermittent or in low numbers on the day of collection whereas prevalence data from longitudinal studies corrects for underestimating by basing prevalence on multiple samples obtained over time (Ralston et al., 2003; Santín et al., 2009). The prevalence of *G. duodenalis* in the present study is similar to prevalences found in Alberta, Canada that included 495 fecal samples from beef pre-weaned calves examined by microscopy (34%) (Appelbee et al., 2003) and in Belgium that included 333 fecal samples from beef pre-weaned calves examined by PCR (45%) (Geurden et al., 2008). Lower prevalences were observed in two studies in Canada both using microscopy as the detection method, the first one reported *Giardia* in 17 and 22.6% of the beef cows and calves in cow-calf herds, respectively (Gow and Waldner, 2006); the second, reported a prevalence of 8.7 and 36% in beef cows and calves (McAllister et al., 2005). A lower prevalence (19.1%) was also reported using microscopy in feedlot cattle from the central and western United States (Hoar et al., 2009). In contrast, Dixon et al. (2011) reported a higher prevalence (72 and 77% by microscopy and PCR, respectively) in beef cattle in Canada. *Giardia*

Table 1
Numbers of calf fecal samples examined and assemblages of *Giardia duodenalis* found by region.

Region	Number of operations	No. samples per operation	No. positives per operation	Prevalence	*Giardia duodenalis* assemblages identified
Midwest	20	20	17	85	E (16) A(1)
		20	17	85	E (17)
		20	7	35	E (6) A (1)
		20	11	55	E (11)
		20	10	50	E (10)
		20	10	50	E (8) A (2)
		20	9	45	E (9)
		20	8	40	E (8)
		20	8	40	E (8)
		19	10	52.6	E (10)
		18	2	11.1	E (2)
		17	6	35.3	E (5) A (1)
		17	1	5.9	E (1)
		16	8	50	E (8)
		16	14	87.5	E (6) A (4) Mix (4)
		15	10	66.7	E (10)
		12	1	8.3	E (1)
		11	2	18.2	E (2)
		8	4	50	E (4)
		8	0	0	
Region total		337	155	46	E (142) A (9) Mix (4)
South	15	20	6	30	E (6)
		20	7	35	E (5) E-like (2)
		20	1	5	E (1)
		20	5	25	E (5)
		20	0	0	
		20	0	0	
		20	1	5	E (1)
		19	0	0	
		18	7	38.9	E (7)
		17	1	5.9	E (1)
		14	0	0	
		10	1	10	E (1)
		8	4	50	E (4)
		3	3	100	E (3)
		3	0	0	
Region total		232	36	15.5	E (34) E-like (2)
West	14	20	1	5	E (1)
		20	6	30	E (6)
		20	3	15	E (3)
		20	18	90	E (18)
		20	8	40	E (8)
		20	4	20	E (4)
		20	3	15	E (3)
		20	7	35	E (7)
		20	11	55	E (11)
		20	4	20	E (4)
		17	4	23.5	E (4)
		17	7	41.2	E (7)
		9	7	77.8	E (6) A (1)
		7	0	0	
Region total		250	83	33.2	E (82) A (1)
Total	49	819	274	33.5	E (258) E-like (2) A (10) Mix (4)

prevalence varies substantially between studies depending on the sampled population and laboratory methodology, usually with higher numbers of positive animals reported in studies in which detection was conducted by PCR versus microscopy. Longitudinal studies have demonstrated that following animals over time will affect prevalence considerably and that a 100% prevalence is common in this type of studies in dairy and beef cattle (Ralston et al., 2003; Santín et al., 2009).

Based on molecular characterization using the SSU rRNA gene two assemblages, assemblages A and E, were identified among the 274 PCR positive fecal samples examined.

Most of the *Giardia* isolates (258) belonged to assemblage E representing 94.2% of the *Giardia*-positives. Assemblage A, a mixed infection with assemblage A/E, and assemblage E-like were only identified in 10, 4, and 2 samples representing 3.6, 1.5, and 0.7% respectively among the *Giardia*-positives. Previous studies have also shown assemblage E to be the predominant assemblage, being found in 80–100% of the specimens in dairy and beef cattle (O'Handley et al., 2000; Trout et al., 2004, 2005, 2006, 2007; Santín et al., 2009; Dixon et al., 2011). For example, only assemblage E was identified in beef cattle farms in Canada (Dixon et al., 2011). Mixed infections with A/E in 2 dairy cattle from Georgia were also reported by Feng et al. (2008). Although assemblage B has been identified in cattle (Lalle et al., 2005), assemblage B was not identified in any of the animals studied in our survey.

5. Conclusions

This study demonstrated substantial levels of *G. duodenalis* infection in weaned beef calves. This level appears to be sufficient to provide a reservoir of infectious organism to new calves. Molecular characterization demonstrated that only a minority of the calves harbored the potentially zoonotic assemblage A while most calves were infected with the livestock-specific assemblage E. Although the prevalence of *G. duodenalis* assemblage A appears low, calves in cow-calf operations should be considered as potential sources of human infective cysts.

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